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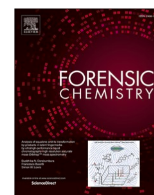

Ante- and post-mortem human volatiles for disaster search and rescue

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ABSTRACT

Ante-mortem metabolic processes are responsible for the release of volatile organic compounds, which form the primary component of human scent and are used by search-and-rescue canines in victim location efforts. Similarly, the post-mortem processes of autolysis and putrefaction produce malodourous compounds that cadaver detection dogs use to locate human remains. This review examines literature on ante-mortem and post-mortem volatiles, with a focus on studies from 2010 onwards. A total of 973 different compounds were reported over this period, from the live matrices blood (65), breath (124), fingernails (17), hair (24), saliva (343), skin (385), sweat (37), urine (80), the whole body (86), and unspecified sources (31), and during early decomposition (321), middle decomposition (49), late decomposition (102), and an unspecified timeframe (113). There are notably more studies examining the matrices from living volunteers than decedents, and methods vary significantly between studies on living and deceased individuals in sampling methodology and analytical instrumentation. To establish a profile that accurately reflects the whole human volatilome, the standardisation of methodology and further research are required. Determining the complete human odour profile will assist in victim location where living and deceased individuals are commingled (*e.g.* disaster sites), and will inform future technologies to aid in accelerating search-and-rescue operations.

1. Introduction

Organisms are constantly releasing a series of volatile organic compounds (VOCs), which are perceived as odours and comprise the VOC profile of an individual [1–5]. VOCs are dynamic, low molecular weight compounds emitted by all organisms, and are released through biological processes during life and through the decomposition of cells post-mortem [6–12]. The metabolic, abiotic, and biotic processes of a living organism contribute to the formation and release of VOCs, which are collectively referred to as the VOC profile, or the volatilome [13]. The volatilome is suspected to be unique among individuals, with differentiation occurring due to primary (genetic), secondary (diet and environment), and tertiary (external additions such as perfume) influential factors [13,14,7].

In the living, VOC studies are often conducted to gain insight into the biochemical processes that occur between healthy and unhealthy individuals. Reported VOC classes emitted from breath [10,15–17], saliva [18–20], skin [21–23], and the whole-body [24–28] include alcohols, aldehydes, aliphatic and aromatic hydrocarbons, ketones, halides, carboxylic acids, ethers, esters, sulfur-containing, and nitrogen-containing compounds [24,29,30,8]. A large portion of human scent studies perform matrix sampling, which include blood [31–33], urine [4,32–38], sweat [39,40], saliva [18–20,32,33,41], and breath [10,16,42–45] analysis. In these instances, VOCs are used to trace disease progression through biomarker identification [16,46–49], analyse cell lines [8], for pollutant and toxin studies, microorganism activity analysis, and for the distinction of chemical markers within the human profile as a potential source of individual human identification [4,8,10,14,28,50].

In decomposing organisms, VOCs are produced through the processes of autolysis and putrefaction, which consist of the self-digestion of cells and subsequent breakdown of macromolecules (proteins, lipids, carbohydrates, and nucleic acids). These macromolecules are degraded by bacterial enzymes [3,51,52], producing VOCs as by- and end-

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products including alcohols, hydrocarbons, ketones, carboxylic acids, esters, ethers, nitrogen- containing, and sulfur-containing compounds. VOC studies of decedents provide insight into the post-mortem chemical processes [3,53,54], which can be applicable in forensic investigations by using the human volatilome as a training aid for cadaver detection dogs as well as increasing the accuracy of post-mortem interval (PMI) estimations [2,3,6,12,51,54].

Although studies of both living and deceased humans have been well documented, the change in VOC classes and concentrations between the ante- and post-mortem states have not been thoroughly explored. There are several potential uses of this information, including the detection of missing persons whose status may be unknown, or where living and deceased individuals are present at the same location, such as during mass disaster events. This review therefore compares the VOCs emitted by living volunteers and human remains to identify the primary contributors to each respective VOC profile. Emission sources of ante-mortem volatiles include breath, skin, and the whole body, and although the odour of biological fluids can be considered separate from living human scent, volatiles from blood, urine, saliva, and sweat have been included within the ante-mortem profile. This is due to their applicability to search-and-rescue operations, as their presence is typically indicative of human life. While there are numerous applications of VOC studies, this review will be focusing on the forensic uses of volatile profiling from 2010 onwards, and in particular its use in locating missing persons and mass disaster victims.

When searching for publications within the scope of this review, the search terms “volatile organic compound” or “VOC” were deemed necessary in all articles, as were synonyms of “odour”. The terms “post-mortem” and “decomposition” were used interchangeably to find research on human remains, and a focus was placed on post-mortem studies using human donors instead of surrogate research. When looking for ante-mortem literature, studies focusing on different matrices were found using words such as “breath”, “urine”, or “skin”. Ante-mortem studies including words such as “cancer” were excluded, as these are prevalent within the medical field and focus on using VOCs for disease diagnoses and not forensic purposes [55]. These search terms were used in conjunction with common search and Boolean operators to better find literature within the scope of the review. Several databases were used to find publications, including ScienceDirect, PubMed, Scopus, JSTOR, Scopus, Google Scholar, and the UTS Library database.

2. Volatile collection techniques

Volatiles can be collected using a variety of different techniques which employ both passive and active sampling methods. Active sampling uses a pumping device to pass air from the headspace (HS) above a specimen onto the sampling medium, whereas passive sampling relies on the movement of gas phase molecules across a concentration gradient to contact the sorbent material and establish an equilibrium [56–58]. In both active and passive sampling, the mechanisms of VOC adsorption include partitioning, electrostatic attraction, and polarity-dependent interactions between hydrophobic/hydrophilic sites [59]. The more readily a compound is adsorbed to the sorbent, the greater the compound’s affinity, which varies between sorbent types depending on sorbent pore size, volume, and polarity [59,60]. In highly concentrated samples or during prolonged sampling periods, compounds with a higher affinity will remove compounds with a lower affinity from the sorbent. This can mean some compounds do not appear during analysis and skew results, which can be a potential source of inaccurate data from both active and passive sampling.

The most common method of passive sampling is solid-phase microextraction (SPME), which is a sampling technique that employs a silica fibre coated with a polymeric sorbent or an immobilised liquid [61]. SPME has several advantages, including its low cost, low uptake of water, high sensitivity, reduced sampling time, and its ease of use. As such, it is often utilised for the *in vitro* study of VOC production [8,11].

SPME has been applied in ante-mortem odour research to profile the VOCs from breath [32,33,62], skin [14,21,32,33,41,63–71], hair [41], fingernails [41], saliva [19,20,32,33,41], blood [32,33], and urine [32,35,36,4,72] (Table 1). SPME is often used for ante-mortem studies where the matrix can be collected and stored (*e.g.* urine, sweat, or saliva), as the sample can be easily collected and analysed in a laboratory setting. Different sorbent coatings are available for SPME fibres, with the most common for both ante- and post-mortem odour analysis being a combination of divinyl benzene (DVB), carboxen (CAR), and polydimethylsiloxane (PDMS) (Table 1 and Table 2**)**. The largest discrepancy between studies is the sampling time, with fibre exposure times varying from 3 min [35,68] to 21 h [14,32,33,41,70,71] in ante-mortem studies and 10 min [73] to 21 h in decomposition research [71]. These vast inconsistencies in sampling periods indicate that further method development is required, with a focus on sorbent type and sampling time optimisation for various ante-mortem and post-mortem matrices.

SPME is often combined with methods of active collection such as a vacuums or gauze pads, particularly in the analysis of living skin VOCs. Studies by DeGreeff *et al.* [70,71] used a Scent Transfer Unit (STU-100) to move air from within a scent collection chamber onto a gauze pad, which was then placed in a glass vial and sampled for 21 h using a DVB/CAR/PDMS SPME fibre. Gauze pads were also combined with SPME by Curran *et al.* [14,63], who detected acids, alcohols, aldehydes, ketones, hydrocarbons, nitrogen-containing compounds, and acid-esters from the hands of living volunteers. Although SPME is used frequently in laboratory-based studies, the sampling method is less practical during field studies as the fibre is fragile and difficult to seal against contamination [3]. This makes them more suited to sample collection that can be performed in a controlled environment. The sorbent also operates on the principal of competitive binding, which can cause limitations for highly concentrated samples such as decomposing human remains [8]. These issues can be overcome with the use of the newly developed SPME-Arrow, which is a passive sampling device consisting of a steel rod coated with a sorbent medium.

The SPME-Arrow was first trialled in 2015 by Helin *et al.* [74] to sample the volatile amines of wastewater, and have since been utilised in a decomposition study investigating post-mortem microbial emissions [75] amongst other volatile research [76–79]. In 2016 Kresmer *et al.* [80] compared the efficacy of headspace sampling techniques, and found that the SPME-Arrow had higher mass recovery and detection limits of halogens and aromatics than SPME fibres. The SPME-Arrow is advantageous over traditional SPME as it has an increased sorbent volume and is more robust than the fibres, thus making it more applicable for concentrated samples and in situ sampling [74,76,81].

An alternative method to SPME are sorbent tubes or thermal desorption tubes, which are stainless-steel tubes containing a sorbent material that can be amended according to the nature of the targeted VOCs. Due to their robustness and portability, sorbent tubes are the preferred method of collecting samples *in situ* [3,6], and can be used to collect VOCs both actively or passively. Employing active sampling enables the collection rate and time to be controlled, thus allowing for consistent sample sizes across a trial and between studies [82]. Combining sorbent tubes with a pump to actively collect VOCs is the most common method of sampling the decomposition volatilome [11] (Table 2), and the large sorbent volume in comparison to SPME fibres also results in a decreased risk of saturation in concentrated scenarios [83]. The most common flow rate is 100 mL/min for post-mortem research (Table 2), however Focant *et al.* [53] and Paczkowski *et al.* [84] sampled at rates of 20–1000 mL/min and 70 L/h (1166 mL/min) respectively, illustrating the large variability of this parameter. The sorbent type in thermal desorption tubes is also changeable, and in post-mortem research they typically contain a combination of Tenax® TA [12,85–93], Carbograph 5TD [85,86,94,95], Tenax® GR [1,96], or Carbopack sorbents [97,98]. In 2014 Rosier *et al.* [83] determined Tenax® TA to be more suitable than Carbosieve SIII, Carbotrap 202,

Table 1
Human ante-mortem VOC studies from 2010 onwards, grouped into studies on direct emissions and biological excretions.

Reference	Matrix	Sample size	Sampling medium	Sorbent	Sampling time	Instrument	Column(s)
A. Direct emissions							
2022 Wang *et al.* [45]	Whole body Breath Skin	20	Direct to instrument	–	Volunteers in room for 2.5–3 hrs, with instrument run continuously	PTR-TOFMS	–
2022 Zou and Yang [100]	Whole body sans breath	14: 7F 7M	Sorbent tubes	Tenax® TA DNPH with ozone scrubber	300 mL/min for 13 min 500 mL/min for 13 min	GC–MS	HP-VOC
2020 Mitova *et al.* [25]	Whole body	18: 9F 9M	Sorbent tubes	Anasorb CSC Coconut Charcoal Tenax® TA	1.0 L/min for 2 hrs 30 mL/min	GC–MS GC-FID	DB-WAX Rxi-5MS
2020 Pojmanová *et al.* [122]	Skin (hand)	10: 5F 5M	Glass beads, liquid extraction	–	10 min	GC–MS	SLB-5MS
2020 Wilkinson *et al.* [44]	Breath	–	ReCIVA®	Tenax® GR Tenax® TA/ Carbograph 1TD Tenax® TA/ Carbograph 5TD	200 mL/min for 2.5 min and 5 min	GC–MS	DB-5MS
2020 Zou *et al.* [99]	Whole body sans breath	1M tested twice	Sorbent tubes	Tenax® TA DNPH with ozone scrubber	300 mL/min for 13 min 500 mL/min for 13 min	GC–MS	HP-VOC
2019 He *et al.* [24]	Whole body and breath	14: 6F 8M	Sorbent tubes	Tenax® TA	300 mL/min for 10 min	GC–MS	HP-VOC
2018 Duffy *et al.* [65]	Skin (hand)	8: 4F 4M	Direct SPME	50/30 µm DVB/ CAR/PDMS	15 min	GC–MS	SLB-5MS
2018 Tsushima *et al.* [43]	Breath and skin vs whole body	5: All M	Sorbent tubes Silica cartridges	Tenax® TA/ activated charcoal DNPH	220 mL/min for 9 min and 23 min 220 mL/min for 40 min	GC–MS and HPLC-DAD	RTX-624
2017 Colón-Crespo *et al.* [66]	Skin (hand)	105: 54F 51M	SPME of gauze pad	50/30 µm DVB/ CAR/PDMS	15 hr	GC–MS	HP-5MS
2017 Doležal *et al.* [123]	Skin (hand)	9F	Glass beads, liquid extraction	–	10 min	GC×GC-TOFMS	[1]D ZB-5MS [2]D BPX-50
2017 Grabowska-Polanowska *et al.* [124]	Skin (forearm)	–	Cellulose film bag + sorbent tube Cellulose sachet filled with sorbent	Carbotrap X (40/60 mesh) Activated carbon	40 min A, 800 mL S Placed on skin for 2 hr	GC–MS	DB-1
2017 Sakumura *et al.* [10]	Breath	29 healthy	Analytic barrier bag	–	Unknown	GC–MS	DB-1
2016 Bigazzi *et al.* [16]	Breath	3M	ATD cartridges	Tenax® TA/ Carbotrap B	50–75 mL/min to make 2 L total	GC–MS	DB-VRX
2016 Caraballo *et al.* [67]	Skin (hand)	20: 10F 10M	Gauze pad STU-100 onto gauze pad Stainless steel bar, then STU-100 onto gauze pad Kodak photo paper, then STU-100 onto gauze pad (All gauze pads sampled using SPME)	50/30 µm StableFlex DCV/ CAR/PDMS	Held for 10 min STU-100 operated for 1 min Held for 5 min, then STU-100 operated for 1 min Held for 5 min, then STU-100 for 1 min	GC–MS	DB-225
2016 Tang *et al.* [125]	Whole body	Class-room	Direct to instrument	–	5 min	PTR-TOFMS	–
2015 Liu *et al.* [26]	Whole body	Class-room	Direct to instrument	–	–	PTR-MS	–
2014b Mochalski *et al.* [22]	Whole body sans breath	10: 3F 7M	Direct to instrument	–	50 mL/min	SRI-TOFMS	–
2014a Mochalski *et al.* [21]	Skin (hand and forearm)	31: 15F 16M	Put arm in Nalophan bag and drew HS with a syringe Used SPME fibre to sample headspace from syringe	75 µm CAR-PDMS	30 min A, 50 mL sample 25 min exposure	GC–MS	PoraBond Q
2013 Brown *et al.* [41]	Fingernails Hair Skin (hand)	20	SPME SPME SPME of gauze pad	50/30 µm DVB/ CAR/PDMS	Equilibrated for 24 hr, sampled for 21 hr Held for 10 min, sampled for 21 hr	GC–MS	HP-5MS

(continued on next page)

Table 1 (*continued*)

Reference	Matrix	Sample size	Sampling medium	Sorbent	Sampling time	Instrument	Column(s)
2013 Dormont *et al*[68]	Skin (foot)	Two trials: 10 adults – 5F 5M; 16 children –	Razor scraped on feet then underwent solvent extraction	–	Scraped on skin for 20 s, then extracted for 2 min	GC–MS	WCOT CPSil-8CB
		7F 9M	SPME on feet in Nalophan bag SPME fibre stroked on skin	1: 65 µm PDMS-DVB 2: 50/30 µm PDMS/DVB/CAR	45 min 3 min		
			Sorbent tubes	Carbotrap B/Tenax® TA	5–20 min at 300 mL/min		
2013 Giannoukos *et al.* [27]	Whole body	2: 1F 1M	Direct – from chamber	–	0.1–1.1 L/min	MIMS-QMS	–
2013 Kusano *et al.* [32,126]	Breath	31: 16F 15M	SPME of Teflon Bio-VOC® breath sampler	50/30 µm DVB/CAR/PDMS	21 hr exposure	GC–MS	HP-5MS
	Skin (hand)		SPME of gauze pad		Held gauze for 10 min SPME exposure not specified		
2012 Ruzsanyi *et al.* [23]	Skin (stomach)	7: 1M 6M	Direct	75 µm CAR/PDMS	2–3 mL/min for 5 min; SPME exposed for 10 min	GC-IMS	PoraBond Q
2011 Prada *et al.* [69]	Skin (hand)	6: 3F 3M	Direct onto fabric, STU-100 onto fabric, then fabric under SPME	DVB/CAR	21 hr exposure	GC–MS	HP-5MS
2011 DeGreeff *et al.* [70]	Skin (hand)	4: 2F 2M	STU-100 and gauze pads, SPME of pads	DVB/CAR/PDMS	60 s collection, 21 hr exposure	GC–MS	HP-5MS
2011 DeGreeff and Furton [71]	Skin (hand)	8: 4F 4M	STU-100 and gauze pads, SPME of pads	DVB/CAR/PDMS	60 s collection, 21 hr exposure	GC–MS	DB-225MS
2011 Kusano *et al.* [33]	Breath	Not specified	SPME of 150 mL Teflon®-bulb	DVB/CAR/PDMS	21 hr exposure	GC–MS	HP-5MS
2010 Curran *et al.* [14]	Skin (hand)	10: 5F 5M	Gauze pad, SPME of pad	DVB/CAR	Held pads for 10 min, 21 hr exposure	GC–MS	HP-5MS
B. Biological excretions							
2019 Erb *et al.* [37]	Urine	Approx. 90 M	Direct HS injection	–	Urine heated for 30 min	GC–MS	DB-5MS
2017 Dubois *et al.* [31]	Blood	3 volunteers, 27 samples (different time points)	Sorbent tubes	Tenax® GR/Carbograph B	200 mL/min for 10 min	GC×GC-TOFMS	^{1}D Rtx-Sil MS ^{2}D Stabilwax
2013 Al-Kateb *et al.* [18]	Saliva	10: 3F 7M	Direct	–	–	GC–MS	ZB-624
2013 Brown *et al.* [41]	Saliva	20	SPME on swab	50/30 µm DVB/CAR/PDMS	Swabbed for 30 s, sampled for 21 hr	GC–MS	HP-5MS
2013 Kusano *et al.* [32,126]	Blood	31: 16F 15M	SPME of Whatman FTA® MiniCard	50/30 µm DVB/CAR/PDMS	18 hr exposure	GC–MS	HP-5MS
	Saliva Urine		SPME of buccal swab SPME		21 hr exposure 30 min exposure		
2013 Kwak *et al.* [36]	Aging urine	6: 3F 3M	SPME	50/30 µm DVB/CAR/PDMS	30 min exposure	GC–MS	Stabilwax
2013 Liu *et al.* [39]	Sweat (armpit, neck, and forehead)	1	SPME on gauze	50/30 µm CAR/DVB/PDMS	–	GC–MS	DB-WAX
2012 Martin *et al.* [19]	Saliva	2 trials: 1F for 6 samples (5 min); 5F 5M for SPME and passive drool	SPME cartridge	PDMS	5 min optimised	GC–MS	DB5
2012c Mochalski *et al.* [4]	Urine	20: 9F 11M	SPME	75 µm CAR/PDMS	45 min optimised	GC–MS	PoraBond Q
2012b Mochalski *et al.* [38]	Urine	1F, 10 samples	Direct HS injection	–	10 mL/min	IMS	MCC, OV-5, Multichrom
2012a Mochalski *et al.* [35]	Urine	4M	SPME	75 µm CAR/PDMS	3 min exposure	GC–MS	PoraBond Q
2011 Kusano *et al.* [33]	Blood	Not specified	SPME of Whatman FTA® MiniCard	DVB/CAR/PDMS	18 hr exposure	GC–MS	HP-5MS
	Saliva		SPME of buccal swab		21 hr exposure		

(*continued on next page*)

Table 1 (*continued*)

Reference	Matrix	Sample size	Sampling medium	Sorbent	Sampling time	Instrument	Column(s)
	Urine		SPME of urine in glass vial		30 min exposure		
2010 Rudnicka *et al.* [34]	Urine	30: 10F 20M	Direct	–	Headspace sampling	IMS	MCC, OV-5
2010 Soini *et al.* [20]	Saliva	175 (no sex)	Stir bar	PDMS	3 hr at 40*C	GC–MS	DB-5MS

Abbreviations: **Sample size** – F (Female), M (Male); **Sampling medium** – SPME (solid-phase microextraction), ATD (adsorption/thermal desorption); STU-100 (scent transfer unit), HS (headspace), FTA (Flinders Technology Associates); **Instrument** – PTR-TOFMS (proton-transfer-reaction with time-of-flight mass spectrometry), GC–MS (gas chromatography-mass spectrometry), GC×GC-TOFMS (two-dimensional gas chromatography with time-of-flight mass spectrometry), HPLC-DAD (high-performance liquid chromatography with diode-array detection), SRI-TOFMS (selective reagent ionisation with time-of-flight mass spectrometry), MIMS-QMS (membrane inlet mass spectrometry with quadrupole mass spectrometry), GC-IMS (gas chromatography with ion mobility spectrometry); **Column(s)** – ^{1}D (first dimension), ^{2}D (second dimension).

Carbopack B, Tenax®/Unicarb, and Tenax®/Carboxen 1003/Carbopack B for sampling post-mortem VOCs, and Tenax® TA sorbent tubes are the most commonly used tubes in both decomposition research and ante-mortem studies [24,25,31,43,99,100]. Although active sampling offers a distinct advantage over passive sampling, it has the potential to overload the sorbent, and caution must be applied when establishing the sampling parameters. Once the volatiles are collected either passively or actively, the sorbent tubes are heated to unbind the compounds from the sorbent material, which is a process called thermal desorption (TD) and is performed prior to instrumental analysis.

To determine the best method of sampling decomposition odour in soil, Perrault *et al.* [101] collected the headspace of soil samples near decomposing pig remains using both SPME fibres (PDMS/DVB) and sorbent tubes (Tenax® TA/Carbograph 5TD). The study found that sorbent tubes collected more decomposition-specific VOCs such as nitrogen-containing compounds, alcohols, hydrocarbons, and sulfides, which indicates that sorbent tubes are the more suitable sampling medium for profiling decomposition. Sorbent tubes are heavily utilised in decomposition studies (Table 2), however are underrepresented in ante-mortem research in favour of SPME and direct headspace injection (Table 1). This makes the comparison between ante- and post-mortem volatile profiles difficult as the methodology between the two areas remains inconsistent.

Although SPME and sorbent tubes are the most common sampling techniques, there are several emerging methods being used to sample VOCs within the food and medical fields [102–104]. One method is hi-capacity sorptive extraction probes (HiSorb), which are used to collect VOCs from liquids or sample headspaces. HiSorb probes were developed to overcome the fragility and cost of SPME fibres [103], and have been found to perform better than SPME fibres when analysing less volatile compounds [102]. The probes can also be used in conjunction with sorbent tubes, and offer promising results in faecal and urine sampling [103,104]. Another approach to volatile sampling is thin-film SPME (TF-SPME), which consists of a carbon mesh strip impregnated with a sorptive phase that is used by inserting the strip into a vial along with the sample [105]. TF-SPME has recently been used to analyse volatiles from water containing human remains [85], where the efficacy of sorbent tubes and TF-SPME were compared. The sorbent tubes yielded a higher diversity of compounds including alcohols and sulfur-containing compounds, although a distinct advantage of TF-SPME is that it can be submerged in the water to sample volatiles directly. TF-SPME has also been applied to sample saliva metabolites [106], with applications in roadside testing and disease detection. Both TF-SPME and HiSorb probes can be used in combination with other methods such as multi-cumulative trapping, where multiple samples are extracted onto the instrument cold trap before sample desorption [107]. This addition is useful when analysing samples of low concentrations, however using the technique with concentrated samples could overload the cold trap, instrument column(s), or detector, so caution should be applied to its use.

The analysis of VOCs is performed through a variety of techniques, the most common of which is gas chromatography (GC). GC involves the injection and transport of analytes via a carrier gas onto a column coated with a stationary phase. Compounds are separated by their mass and polarity, as the affinity of these analytes for the stationary phase versus the mobile phase determines their elution time [108,109]. As this method requires compounds to be in their gas phase, the low molecular weight and boiling point of VOCs makes GC an ideal separation technique [110]. GC is typically coupled with mass spectrometry (GC–MS), which allows mass spectral information to be collected for each analyte. GC–MS has been used to analyse the VOCs produced through human [9,111–114] and animal decomposition [2,84,115] (Table 2), as well as from ante-mortem matrices including skin/sweat [116,14,22–24,26,43,63,64,69–71,7], breath [10,16,24,43,62,117], urine [4,34,38,62,72], saliva [18,20,23,118], and faeces [119] (Table 1). Despite its popularity, GC–MS is unable to separate co-eluting compounds, which masks trace VOCs and is hence problematic for complex ante- and post-mortem samples [91]. To overcome this issue, two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS) is now the preferred method for complex mixture analysis, as co-eluting compounds can be separated in the second dimension [108,120,50,51,53,97], and the technique enables enhanced peak capacities and increased mass spectral sensitivity [51,91,97,109,120].

Table 2 summarises the collection and analysis techniques that are most commonly employed in VOC studies concerning cadaveric decomposition. These techniques are not specific to decomposition and can also be applied to living organisms. Thermal desorption with GC×GC-TOFMS has proven to be the most effective technique in decomposition volatile collection and analysis [91], however the standardisation of sampling methodology and analysis techniques is required for the accurate comparison of volatilomes. In addition to these measures, a combination of using standard test mixes, specialised alignment software, and retention time locking [121] should be employed to account for variation in analyte retention times between systems. Currently most ante-mortem studies have been conducted using one-dimensional (^{1}D) gas chromatography (GC) (Table 1), which complicates the comparison between ante- and post-mortem studies as the results are collected using different separation mechanisms. Various compounds and compound classes may therefore be underrepresented in the ante-mortem profile, as compounds may be co-eluting or at concentrations too low for the technique to detect.

3. Ante-mortem volatile organic compounds

Volatilome profiling studies can enable the detection of human chemical signatures, and can provide information on the biochemical process occurring within an individual [28,117]. Applications of this research includes potential victim identification and location in search-and-rescue operations, as these rescue operations are frequently carried out by scent-detection canines due to their ability to accurately detect and trail scent [4,22,28].

The volatilome of living humans is created through the metabolic processes in various tissues (*e.g.* lungs, skin, gastrointestinal tract), with skin and biological fluids (*e.g.* sweat, urine, saliva) emitting the

Table 2
Summary of decomposition VOC studies, separated into studies using human donors and animal remains.

Reference	Specimen	Deposition site	Sampling medium	Sorbent	Sampling time	Instrument	Column
A. Studies using human donors							
2024 Ho *et al.* [85]	Amputated human foot (1)	Submerged in large tank of water indoors	Sorbent tubes TF-SPME	Tenax® TA/ Carbograph 5TD PDMS/DVB	100 mL/min for 5 min 5 min	GC×GC-TOFMS	^{1}D Rxi-624Sil MS ^{2}D Stabilwax
2024 Schieweck *et al.* [94]	Human (9) in different stages of decomposition	In body bags within an autopsy room	Sorbent tubes	VVOCs: Carbograph 5TD VOCs and odour analysis: Tenax® TA	125 mL/min for 4–32 min, depending on decay stage	VVOCs and VOCs: GC–MS Odour analysis: GC-O	VVOCS: DB 624 VOCS: DB5-MS Odour analysis: HP-5 MS
2024 Thurn *et al.* [86]	Human (2)	Surface deposits in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 10 min	GC×GC-TOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2023 Martin and Verheggen[127]	Human (20)	In body bags within a morgue	Sorbent tubes	Tenax® TA/ Carbograph	200 mL/min for 10 min	GC–MS	Rtx-5 MS
2023 Patel *et al.* [128]	Human (8)	In shroud within a morgue	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 10 min	GC×GC-TOFMS	^{1}D Rxi-624Sil MS ^{2}D Stabilwax
2021 Ueland *et al.* [95]	Human (12)	Surface deposits in an open eucalypt woodland, Australia, covered with rubble and debris	Sorbent tubes	Tenax® TA/ Carbograph 5TD	Passive collection for ≥ 24 hrs	GC×GC-TOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2019 Deo *et al.* [12]	Human (5)	Surface deposits in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 10 min	GC×GC-TOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2019 Dubois *et al.* [96]	Human (5) tissue from kidney, liver, lung, heart, and blood	Glass vials in a laboratory, Belgium	Sorbent tubes	Tenax® GR/ Carbograph B	200 mL/min for 10 min	GC×GC-TOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2017 Rosier *et al.* [88]	Tissue and organs of humans (5)	Glass jars in a laboratory environment	Sorbent tubes	Tenax® TA	100 mL/min for 20 min	GC–MS	VF-625 MS
2016 Rosier *et al.* [90]	Tissue and organs of humans (6)	Glass jars in a laboratory environment	Sorbent tubes	Tenax® TA	100 mL/min for 20 min	GC–MS	VF-625 MS
2016 Stefanuto *et al.* [129]	Human (4)	Not specified	SPME	PDMS	15 min fibre exposure	GC×GC-HRTOF-MS	^{1}D Rxi-5Sil ^{2}D Rxi-17Sil MS
2015 Rosier *et al.* [89]	Tissue and organs of humans (6)	Glass jars in a laboratory environment	Sorbent tubes	Tenax® TA	100 mL/min for 20 min	GC–MS	VF-625 MS
2015 Stefanuto *et al.* [98]	Human (4)Pig (2)	Surface deposits in grassland TX, USA	Sorbent tubes	Tenax® GR/ Carbopack B	200 mL/min for 5 min	GC×GC-TOFMS	^{1}D Restek Rxi-5Sil ^{2}D Restek Rxi-17
2012 Boumba *et al.* [130]	Human blood	Taken by pathologist during autopsies	Direct HS injection	–	500 µL of headspace	GS-GC-FID	Supelcowax
2012 Vass[113]	Soil (1 8 6) from human gravesites	Soil samples in glass 40 mL VOC vials	Direct HS injection	–	2 mL of headspace	GC–MS	Restek Crossbond Rtx-1PONA
2011 DeGreeff and Furton[71]	Human (27) Animal (8)	21 in a morgue 6 in a crematorium Various depositions	STU-100 and gauze pads, then SPME	DVB/CAR/PDMS	STU-100 for 60 s, then 21 hr fibre exposure	GC–MS	DB-225MS
B. Studies using human analogues							
2019 Irish *et al.* [115]	Pig (6)	Plastic boxes – 3 in air, 3 submerged in water	SPME	PDMS/CAR/ DVB	40 min fibre exposure	GC–MS	HP-5MS
2019 Martin et al [131]	Rat (12)	Glass vivariums with sand, wood chips, polystyrene, and sugar	Tenax® TA cartridge	2,6-diphenylen oxide	–	GC–MS	VF-624 MS
2019 Xia *et al.* [132]	Skeletal muscle of rats (1 1 0)	Glass bottles in a laboratory environment	SPME	DVB/CAR/ PDMS	30 min fibre exposure	GC–MS	HP-5 MS
2017 Nizio *et al.* [73]	Pig (6)	Surface deposits in open eucalypt woodland, Australia	SPME of fabric swatches	DVB/CAR/ PDMS	10 min fibre exposure	GC×GC-TOFMS	^{1}D Rxi-624Sil MS ^{2}D Stabilwax
2017 Rosier *et al.* [88]	Tissue and organs of pigs (4), lambs (2), and roe (1)	Glass jars in a laboratory environment	Sorbent tubes	Tenax® TA	100 mL/min for 20 min	GC–MS	VF-625 MS
2016 Armstrong *et al.* [133]	Pig (3)	Surface deposits in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 10 min	GC×GC-TOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2016 Forbes *et al.* [134]	Pig (2)	Burials in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 15 min	GC×GC-TOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2016 Rosier *et al.* [90]	Tissue and organs of mammals (9), fish (1),	Glass jars in a laboratory environment	Sorbent tubes	Tenax® TA	100 mL/min for 20 min	GC–MS	VF-624MS

(*continued on next page*)

Table 2 (*continued*)

Reference	Specimen	Deposition site	Sampling medium	Sorbent	Sampling time	Instrument	Column
	amphibians (4), reptiles (1), and birds (11)						
2014 Paczkowski *et al.* [84]	Pig (4)	Surface deposits in a deciduous forest, Germany	Sorbent tubes	Tenax®	1166 mL/min for 120 min (70 L/hr for 2 hr)	GC–MS	HP-5MS
2015 Perrault *et al.* [93]	Soil under pigs (4)	Surface deposits in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 15 min	GC×GC-TOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2015 Perrault *et al.* [91]	Pig (4)	Surface deposits in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 15 min	GC×GC-TOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2015 Perrault *et al.* [92]	Soil under pigs (4)	Surface deposits in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 15 min	GC×GC-TOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2015 Rosier *et al.* [90]	Tissue and organs of mammals (9), fish (1), amphibians (4), reptiles (1), and birds (11)	Glass jars in a laboratory environment	Sorbent tubes	Tenax® TA	100 mL/min for 20 min	GC–MS	VF-624MS
2015 Stefanuto *et al.* [1]	Soil under pigs (4)	Burials in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 30 min	GC×GC-HRTOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2014 Forbes *et al.* [3]	Pig (4)	Surface deposits in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 10 min	GC×GC-TOFMS	^{1}D Rxi-624Sil ^{2}D Stabilwax
2014 Forbes and Perrault[2]	Pig (4) and soil	Surface deposits in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	Pigs: 100 mL/min for 10 min Soil: 100 mL/min for 30 min	GC–MS	DB-VRX
2014 Perrault *et al.* [101]	Soil under pig (4)	Surface deposits in open eucalypt woodland, Australia	Sorbent tubes	Tenax® TA/ Carbograph 5TD	100 mL/min for 30 min	GC–MS	DB-VRX
			SPME	PDMS/DVB (65 um)	20 min at 40 °C		
2014 Stadler *et al.* [135]	Pig (3 trials, 4 per trial)	Surface deposits in open grassland, Canada	Sorbent tubes	Tenax® GR/ Carbopack B	200 mL/min for 5 min	GC–MS	Varian VF-5MS
2014 Stefanuto *et al.* [97]	Pig	Surface deposit in open grassland, Canada	Sorbent tubes	Tenax® GR/ Carbopack B	200 mL/min for 5 min	GC×GC-TOFMS	^{1}D Rxi-5Sil ^{2}D Rxi-17Sil
2013 Focant *et al.* [53]	Pigs and soil	Surface deposit in a forest biotype, Belgium	Absorbent filter	40 ug SuperQ	200–1000 mL/ min for 0.5–1 hr	GC×GC-TOFMS	^{1}D Rxi-5Sil ^{2}D BPX-50
			Sorbent tubes	Tenax® GR/ Carbopack B	200 mL/min for 5 min		
2012 Brasseur *et al.* [136]	Pig (3 cycles, 2 per cycle)	Burials in a forest biotype, Belgium	Adsorbent filter	60 ug SuperQ absorbent filter (80–100 mesh)	500 mL/min for 1–2 hr	GC×GC-TOFMS	^{1}D VF-1MS ^{2}D VF-17 MS
2012 Cablk *et al.* [137]	Cow, pig, and chicken	Tissue samples in 20 mL glass sample vials	SPME	PDMS/DVB	20 min	GC–MS	DB5-MS
2012 Dekeirsschieter *et al.* [120]	Pig (1)	Surface deposit in a forest biotype, Belgium	Adsorbent filter	40 ug SuperQ adsorbent filter (80–100 mesh)	1000 mL/min for 1 hr	GC×GC-TOFMS	^{1}D Rtx-5 ^{2}D Rxi-17
2012 Kasper *et al.* [138]	Mice (60)	Glass petri dish in climatic exposure test cabinets (one 22 °C, 80–90 % RH, another 12 °C, 40–60 % RH)	Sorbent tubes	Activated charcoal	100 mL/min for 1 hr	GC–MS	DB-Wax
2012 Stadler *et al.* [51]	Pig (2)	Surface deposits in a grassland, Canada	Sorbent tubes	Tenax® GR/ Carbopack B	200 mL/min for 5 min	GC×GC-TOFMS	^{1}D Rxi-5Sil ^{2}D BPX-50
2011 Statheropoulos *et al.* [139]	Pig (3 cycles, 2/cycle)	In a polyethylene body bag, partially buried in 10 kg of rubble	Sorbent tubes	Tenax® TA/ Carbopack X	100 mL/min for 10 min	GC-TOFMS	SPB-624

Abbreviations: **Sampling medium**. SPME (solid-phase microextraction), TF-SPME (thin-film solid-phase microextraction), HS (headspace), VVOCs (very volatile organic compounds); **Instrument** – GC×GC-TOFMS (two-dimensional gas chromatography with time-of-flight mass spectrometry), GC×GC-HRTOF-MS (two-dimensional gas chromatography with high resolution time-of-flight mass spectrometry), GC–MS (gas chromatography-mass spectrometry), GC-O (gas chromatography–olfactometry); **Column(s)** – ^{1}D (first dimension), ^{2}D (second dimension).

produced VOCs. A summary of the volatile compound classes emitted from these matrices can be found in Table 3. The production and abundance of these VOCs changes with the individual, and is dependent on the primary, secondary, and tertiary factors [13,14,7] of genetics, diet, and perfumes respectively. Human scent VOC studies occur primarily through the non-invasive headspace sampling of breath, skin, blood, saliva, and urine. These biological fluids and secretions are continuously emanating hundreds of VOCs, which may allow for a snapshot of metabolic activity to be captured at time of sample collection [7,62]. In addition to its application in search-and-rescue operations, the ante-mortem sampling of volatiles is commonly used in the health sector for disease detection. The presence of diseases such as cancer [10,15,46,55,140] and Parkinson's [141,142] are known to change human volatile emissions, so using VOCs as biomarkers is currently being explored as a potential diagnostic tool. Despite these applications, the present review aims to focus on the forensic uses of volatile emissions, and particularly in the transition from the ante- to post-mortem odour for victim location. As such, VOCs taken from

Table 3
The number of unique compounds per compound class found in ante-mortem studies of human volunteers.

Classes	Blood [31–33]	Breath [10,16,17,32,33,42–45]	Finger nails [41]	Hair [41]	Saliva [18–20,32,33,41]	Skin [122–124,14,147,148,154,21–23,32,41,43,45,65–70]	Sweat [39,40]	Urine [4,32–38]	Whole body [125,22,24–28,43,45,99,100]
Alcohols	10	15	2	2	26	39	1	5	15
Aldehydes	7	10	4	5	36	42	6	9	15
Aromatics	3	12	–	–	17	20	–	8	8
Carboxylic acids	–	3	2	4	17	20	3	5	6
Esters	2	7	2	2	38	100	2	3	9
Ethers	8	5	–	–	10	9	2	3	–
Halogenated	4	4	–	–	2	3	–	5	2
Hydrocarbons	20	31	5	8	68	79	–	1	15
Ketones	3	13	–	–	46	17	12	27	5
Nitrogen-containing	3	8	–	–	9	25	8	4	5
Sulfur-containing	–	7	–	–	10	7	2	6	2
Terpenes	3	7	1	1	45	12	–	2	3
Terpenoids	2	1	1	2	19	3	1	2	1
Others	–	1	–	–	–	–	–	–	–
Grand Total	**65**	**124**	**17**	**24**	**343**	**385**	**37**	**80**	**86**

* The symbol "-" indicates that the compound class has not been reported in that matrix from current ante–mortem studies.

patients with chronic or acute illnesses have been excluded from this review, due to their impact on the volatilome and potential influence on the transition from living to deceased odour.

A similar review by de Lacy Costello *et al.* [30] from 2013 provides a compendium of VOC emitted from a healthy human body, and presents data from the matrices of breath, saliva, blood, milk, skin, urine, and faeces. Breath provided the highest number of VOCs in with 872 compounds, with alcohols, aliphatic and aromatic hydrocarbons, carboxylic acids, esters, ethers, ketones, and nitrogen-, halogen-, and sulfur-containing compounds being represented [30]. It also contained the highest abundance of nitrogen-containing compounds, aromatics, and ethers, with 36 ethers not being found in any other matrix. These VOCs found in exhaled breath originate from biochemical processes within the body, consumed nutrients, and the environment [117]. These findings were compiled from seven studies profiling human breath published from 2005 to 2013, and research published after the review from Kusano *et al.* [32,33], Wang *et al.* [45], Kwak *et al.* [17], Itoh *et al.* [42], Wilkinson *et al.* [44], He *et al.* [24], Tsushima *et al.* [43], Sakumura *et al.* [10], and Bigazzi *et al.* [16] concur with the results. A snapshot of the VOCs produced by ante- and post-mortem matrices from 2010 onwards can be found in Table 5, with the full list available as supplementary material (Table S1). Not all studies included in Table 1 and Table 2 were added to Table S1, as some only reported the total abundance of compound classes and not individual compounds.

The most abundantly detected compound from breath is acetone [16,117,143], which has been found in many biological matrices including faeces [8,119], urine [4,8,28,34,62,72], breath [8,16,24,117], skin [8,22,64,71], and saliva [18–20,118] (Table 3). Acetone is considered endogenous in human metabolism [24,30,144], and is mainly formed through the decarboxylation of acetoacetate and dehydrogenation of isopropanol [4,117]. Acetone is also produced through the decarboxylation of excess acetyl-CoA, which consists of an acetyl unit ligated to coenzyme A [145,146]. Acetyl-CoA is derived from glucose, fatty acid, and amino acid catabolism, and has many roles in metabolic pathways both during times of starvation and high caloric intakes. When the body undergoes starvation, acetyl-CoA is used to synthesise ketones such as acetone, as they act as alternative fuel sources to carbohydrates. Ketones are thus present in higher quantities in the blood and breath of starving individuals, and should be researched further as potential biomarkers for victims in disaster scenarios. The metabolic pathways involving acetyl-CoA, including the production of ketones, can be found in Shi *et al.* [145]. The prevalence of ketones in fasting individuals was also found by Statheropoulos *et al.* [117], who analysed expired air from seven fasting monks. Acetone was the most abundant substance, followed by phenol, limonene, 2-pentone, and isoprene. Other ketones produced in high abundances included 1-phenylethanone, cyclohexanone, and 2-butanone.

Determining the volatile profile of human skin has varied applications, including training detection dogs to identify the scent of an individual [123], determining the attractiveness of odour to mosquitoes [147], and the development of fragrances in the cosmetics field [65]. Skin volatiles are emitted from eccrine, apocrine, and sebaceous glands, which are metabolised by bacteria on the skin to form an individual's unique scent [124]. Eccrine glands produce odourless sweat, and although they are distributed over the whole body, they are concentrated on the hands and feet. Apocrine glands are found solely in the axillae (armpit) and genital regions, and are responsible for the secretion of lipids, proteins, and steroids [29]. Finally, sebaceous glands are concentrated on the head, and produce sebum consisting of cholesterol, cholesteryl esters, squalene, diglycerides and triglycerides, wax esters, and fatty acids (particularly oleic, linoleic, and myristic acids) [23]. Skin primarily produces hydrocarbons and esters as well as alcohols and aldehydes, where aldehydes have been found to be formed from fatty acids through homolytic beta-scission, which is caused by UV radiation or bacterial activity [23]. From the studies taken from 2010 onwards (Table 1) skin produced a total of 385 individual compounds, including

alcohols, aldehydes, aliphatic and aromatic hydrocarbons, esters, ketones, and nitrogen- and sulfur-containing compounds (Table 3 and Table S1). Various VOCs, and particularly aldehydes, are produced from fatty acids in sebum by homolytic β-scission by UV radiation or bacterial peroxisomal lipid oxidation [23]. The aldehyde 2-nonenal has been found to be responsible for the smell associated with the elderly, as its production increases as skin gland composition and secretion changes with age [148,149].

Urine is another prominently analysed matrix, with eleven studies analysing the volatiles found in urine's headspace published between 2005 and 2019 [4,8,28,33–38,62,72]. De Lacy Costello *et al.* [30] determined that 279 compounds were found in the urine of healthy humans, and other urinary studies have documented over 230 VOCs including ketones, aldehydes, hydrocarbons, heterocycles, and sulfur-containing compounds [150,4,5,62,72]. Studies published from 2010 onwards reported 80 compounds, with ketones contributing 27 compounds to the profile (Table 3). Urine is considered an extremely useful source of volatile markers due to the pre-concentration capabilities of the kidneys, and is consequently the most commonly investigated human matrix in forensic search-and-rescue operations [4,150]. In the field of search-and-rescue, Mochalski *et al.* [4] profiled human urine to determine its viability as a source of biomarkers for human presence in disaster scenarios. In this study, headspace samples of urine from 20 healthy volunteers were collected using SPME and analysed using GC–MS. Of the 148 compounds detected, 33 were present in at least 80 % of samples and were considered ubiquitous to urine. These compounds consist of ten ketones, seven aldehydes, seven sulfur-containing compounds, four heterocycles (including two nitrogen-containing compounds), two esters, one terpene, and one aromatic hydrocarbon. The study also examined the evolution of the urinary scent profile over time and determined that the concentrations of 26 of these 33 ubiquitous compounds increased as the experiment progressed. The compounds whose concentration increased the most over a 96 hr period were ketones and sulfur-containing compounds. 4-Heptanone, 3-hexanone, 4-methyl-2-pentanone, and 3-methyl-2-butanone increased between 334–383 %, and methanethiol, dimethyl disulfide, and dimethyl trisulfide increased by 322 %, 499 %, and 525 %, respectively. The concentration of 1-methyl-pyrrole also increased greatly over the four day sampling period by 371 %. The increase in these compounds over time is highly notable, as it determines their potential applicability as biomarkers during disasters where victims may be trapped for several days.

The detection of certain VOCs may be indicative of entrapped live humans, specifically if the VOCs detected are not normally emitted from the environment. However, studies profiling live humans are limited due to the ethical restrictions and inconveniences surrounding placement of humans in confining environmental scenarios [22]. Despite these limitations, Huo *et al.* [151] was able to create a chamber simulating a collapsed building with entrapped casualties. This study was conducted to control and reproduce sampling and monitoring of chemicals emitted within a collapsed structure, representing an environment that could sustain life for a 72-hour period. The study involved participants having to lie down in the chamber over a five-day period where their sweat, breath and skin VOCs were studied as they passed through materials that associated with structural collapse. Although specific VOCs were not reported, it was outlined that some VOCs had rapidly accumulated in the experiment before declining while others were present at significant levels for certain participants while being absent for the remainder of the participants [151]. Another study by Mochalski *et al.* [22] used live volunteers to monitor 12 preselected skin-borne volatiles in an entrapment scenario, which involved the volunteers sitting in an airtight chamber for a one-hour period with a focus on skin VOC emanation. The selected compounds were aldehydes: n-propanal, 2-methyl-2-propenal, n-hexanal, n-heptanal, n-octanal, and n-nonanal; ketones: acetone, 3-buten-2-one, 2-butanone, and 6-methyl-5-hepten-2-one; hydrocarbons: 2-methyl-2-pentene; and the terpene limonene. This research illustrated that generally ketones, in particular acetone, displayed the highest VOC abundances from live volunteers [22]. A similar experimental set-up was conducted by He *et al.* [24] who used an airtight chamber to compare breath and whole body volatilome profiles. This was done in an attempt to address the similarities between these profiles, and to determine whether breath analysis could be utilised and applied in whole human body research applications. Out of the 38 VOCs detected from the whole-body studies by He *et al.* [24], 33 VOCs were also detected in the breath tests. This suggests that the VOC profiles of breath can be considered as partly representative of the whole-body emissions. Acetone and isoprene were found in high concentrations from both breath and whole body samples, and the most frequently detected whole-body VOCs in this study belonged to aldehydes, alkanes, ketones, alcohols, halides, and aromatics [24]. Alkanes were the most abundant compound class produced from the whole-body test, whereas ketones were the most prevalent in the breath tests. This supports the research from Mochalski *et al.* [22], and similar findings have been reported in non-forensic whole-body studies [152,153,63,64].

Due to the deployment abilities and skills of canines to search large disaster scenes in a short time frame, they are more commonly utilised in search-and-rescue situations compared to portable detection instruments [2,9,111,137]. Search-and-rescue canines are trained on a series of human emitted VOCs and then tasked with detecting and locating similar VOC emissions within the scene, with the goal of detecting entrapped humans. The limitations surrounding the application of live scent training includes the fact that many entrapped individuals eventually die in disaster scenarios, and therefore the VOCs that are being emitted may not be applicable or similar to those of a living individual. Furthermore, human matrix scent training can be difficult to implement in forensic search-and-rescue operations due to external influential factors. These include the time and length of entrapment, the scene of entrapment (temperature, type of collapse, humidity etc.), and the medical status of entrapped victims [4]. These influences can challenge the VOCs produced as the concentration of the human markers may be affected and lowered in concentration, therefore making identification and detection of VOCs difficult [4,43].

4. Post-mortem volatile organic compounds

Knowledge of post-mortem VOC production is vital to forensic investigations, as understanding prominent compounds and classes assists in training human remains detection (HRD) canines to ultimately aid in locating missing persons. HRD dogs rely on decomposition odour to locate human remains, clandestine burial sites, and to find victims in mass disaster scenarios, and are trained with samples of decomposition fluid, blood, adipocere, and human tissue [6,11,52–54,95]. They are employed due to their accuracy, sensitivity, and selectivity when searching for human remains, as well as their ability to be rapidly deployed within large search areas [2,54].

Volatiles continue to be emitted from decedents long after their ante-mortem production has ceased [2], and are caused by the processes of autolysis and putrefaction [11,53]. VOCs are produced during all stages of decomposition, however they are mainly formed during putrefaction, where macromolecules are biodegraded by bacterial enzymes, releasing VOCs and causing the distinctive bloating due to the production of gas and fluid. The VOCs produced in relation to macromolecules are summarised in Fig. 1. Gases such as nitrogen, methane, ammonia, and hydrogen sulfide are released [91], and alcohols, carboxylic acids, aromatics, aldehydes, aliphatic hydrocarbons, esters, ketones, and nitrogen- and sulfur-containing compounds, as well as ethers and halogens are produced [2,51,54,91,97,120]. Sulfur-containing compounds are responsible for the "rotten egg-like" odour that is characteristic of decomposing remains [112,137], and have been found to be produced throughout the decomposition process [3,51,111,114] (Table S1).

Human decomposition has traditionally been documented as the following sequence of stages: the fresh stage, bloat, active decay,

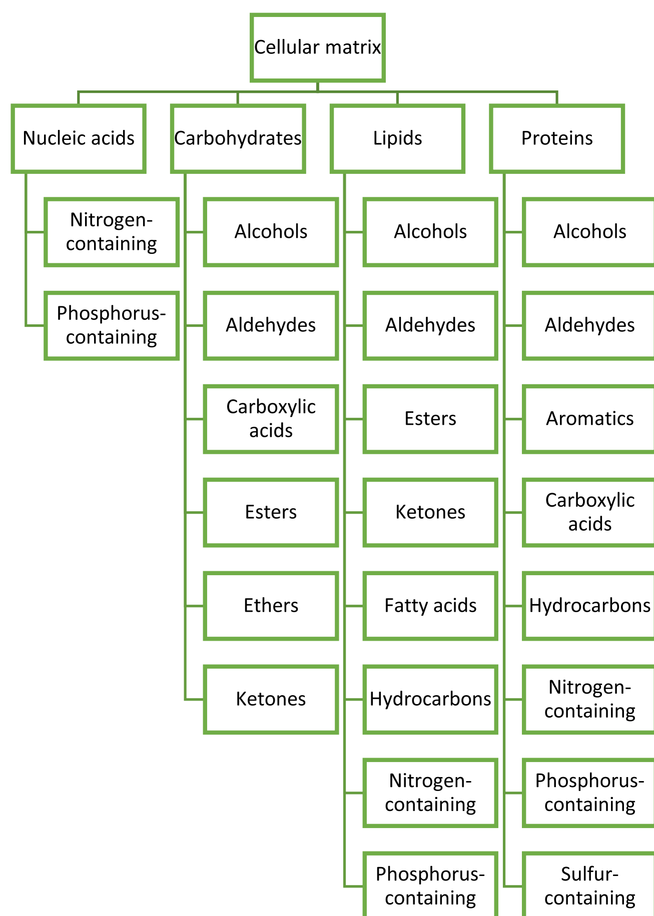

Fig. 1. Breakdown of macromolecules (nucleic acids, carbohydrates, lipids, and proteins) into their product compounds classes [52,54,71,95,112,137,155].

advanced decay, and skeletonization/dry remains [6,11,120,156]. In addition to measuring decomposition through stages, decomposition can also be classed into early, middle, and late time periods. In a study by Deo *et al.* [12], the early stage lasted from 0 to 300 accumulated degree days (ADDs), middle from 300 to 700 ADDs, and late decomposition from 700 ADDs onwards. Of the decomposition stages, the fresh stage shows the lowest emission rates of decomposition VOCs due to the limited amounts of bacterial and microbial activity taking place [2]. A slow increase in VOCs is then reported during the bloat stage, with the maximum abundance and variety occurring during the active and advanced decay stages [120,2,3,9,91]. A decrease in VOC abundance typically occurs as soft tissue is consumed and liquefied via insect and microbial activity. Although VOCs are detected throughout all stages of decomposition, the chemical classes released have been noted to vary depending on the stage of decomposition of the cadaver [91,120] (Table 4 and Table S1).

Ethical restrictions surrounding the studies of human decomposition are one of the major limitations within the field of forensic taphonomy, which has resulted in the use of pig carcasses as human analogues due to their physiological and biochemical similarities with humans [2,51,97,120]. Pigs have a similar gut biota to humans, lack a heavy fur, and have a similar fat-to-muscle ratio, which is why they have been the preferred analogue when studying human decomposition [6]. Although porcine remains can lend some insight into human decomposition, recent work from Knobel *et al.* [6] determined that the rate of decay of the two organisms was not comparable, as pigs decomposed at a steady pace whereas the humans underwent differential decomposition (*i.e.* the concurrence of multiple stages of decomposition within one decedent). This study also found differences in the VOC profiles, in particular the abundance of VOCs during bloat. The humans produced a much higher abundance of aldehydes, aromatics, ethers, and alcohols than the pig remains, which remained consistent across the stages. The inconsistencies between pig and human decomposition also translated into the insect populations, with research from Dawson *et al.* [157] finding large disparities between the fly and beetle populations surrounding the species. Given the known link between insect attraction and carrion volatiles [158–160], it is likely that the differing insect activity is related to variations between the volatile profiles. The number of studies using porcine remains as human analogues is much higher than those using human remains (Table 2), which raises issues when attempting to determine the human volatilome. Furthermore, due to the variation in human internal conditions (including fat content and individual biome), a large number of replicates are required in order to accurately capture the broad VOC profile emitted from decomposing humans. Due to the limited number of VOC studies regarding human decomposition and the inter-variability of VOC produced within humans, implementing these studies becomes challenging. Consequently, a larger database of VOC studies surrounding human decomposition is required to ensure that the greatest variety of VOCs are being encompassed and represented.

5. The transition from ante-mortem to post-mortem odour

One of the biggest challenges in forensic volatilome research is to understand the transition from ante-mortem to post-mortem profiles. Living scent is a combination of volatiles released from sources including skin, breath, and sweat during the ante-mortem period [48], whereas post-mortem odour of humans is comprised of volatiles released

Table 4
The number of unique compounds per compound class found in post-mortem studies of human remains.

Classes	Early [113,128,161,71,86,95,96]	Middle [113,129,71,86,95,96]	Late [113,83,85,86,90,96]	Unspecified [89]
Alcohols	39	3	17	10
Aldehydes	25	9	6	10
Aromatics	17	5	–	14
Carboxylic acids	14	–	6	3
Esters	36	3	18	7
Ethers	6	1	–	2
Halogenated	11	4	–	12
Hydrocarbons	88	10	13	14
Ketones	28	2	15	4
Nitrogen-containing	20	7	7	20
Sulfur-containing	13	2	19	15
Terpenes	11	2	–	1
Terpenoids	7	–	–	1
Others	6	1	1	–
Grand total	**321**	**49**	**102**	**113**

* The symbol "-" indicates that the compound class has not been reported within the time period from current post-mortem studies.

Table 5
An excerpt of the ante-mortem and post-mortem volatiles reported in the literature, with compounds listed under their IUPAC names. The first five compounds per compound class are presented here alphabetically. For the full version with references, see Table S1 in the supplementary material.

Sources	Ante-mortem (AM) sources									Post-mortem (PM) sources			
Compounds	Blood	Breath	Finger nails	Hair	Saliva	Skin	Sweat	Urine	Whole body	Not specified	Early	Middle	Late	Not specified
Alcohols														
1-(1,3-benzodioxol-5-yl)-4,4-dimethylpent-1-en-3-ol														U
1-(2-butoxyethoxy)ethanol									WB					
10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol						Sk								
1-cyclopentyl-2,2-dimethylpropan-1-ol	Bl													
1-methoxypropan-2-ol									WB		E	M		
Aldehydes														
1,2,3-trimethylcyclopent-2-ene-1-carbaldehyde														U
2,4,6-trimethylbenzaldehyde	Bl									U				
2-benzylideneheptanal					Sa	Sk								
2-benzylideneoctanal					Sa	Sk								
2-ethylbutanal						Sk								
Carboxylic acids														
1,2-benzenedicarboxylic acid, 2-ethoxy									WB					
10-methyltridecanoic acid					Sa									
2-aminopentanedioic acid						Sk								
2-ethylbutanoic acid									WB					
2-ethylhexadecanoic acid						Sk								
Esters														
2-ethylhexyl 3-(4-methoxyphenyl)prop-2-enoate						Sk								
(2,4-dimethylphenyl)methyl 3,5-dimethylbenzoate														U
(3,3,5-trimethylcyclohexyl) 2-hydroxybenzoate						Sk								
(3-hydroxy-2,2,4-trimethylpentyl) 2-methylpropanoate									WB					
(3-hydroxy-2,4,4-trimethylpentyl) 2-methylpropanoate						Sk								
Ethers														
(2R,3R)-2-butyl-3-methyloxirane	Bl													
1,1-diethoxyethane											E			
1,3-dioxolane						Sk								
1,4-dioxane		Br									E			
1-methoxy-4-prop-2-enylbenzene					Sa									
Halogenated compounds														
(2,2,2-trichloro-1-phenylethyl) acetate					Sa									
1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)propane											E			
1,1,1-trifluoropropan-2-one											E			
1,1,2,2-tetrachloroethene		Br						U						U
1,1,2-trichloro-1,2,2-trifluoroethane												M		U
Hydrocarbons														
Aromatics														
(3-phenylbut-1-enyl)benzene						Sk								
(4-phenylbut-2-enyl)benzene						Sk								
1,2,3,4-tetrahydronaphthalene		Br												
1,2,3,4-tetramethylbenzene											E			
1,2,3,5-tetramethylbenzene														U
Branched alkanes														
2,2,3,3-tetramethylbutane											E			
2,2,4,4,6,8,8-heptamethylnonane									WB		E			
2,2,4,6,6-pentamethylheptane		Br				Sk					E			
2,2-dimethylbutane						Sk								
2,2-dimethyldecane											E			
Branched alkenes														
2,3-dimethylbut-2-ene						Sk								
2,3-dimethylhex-3-ene											E			
2,4,6-trimethylnon-1-ene											E			
2,4-dimethylpent-2-ene					Sa									
2,5,5-trimethylhepta-1,3,6-triene					Sa									
Cyclic alkanes														
(1R,2S)-1,2-dimethylcyclohexane											E			
(1R,3R)-1,3-dimethylcyclohexane											E			
(1S,2S,4S)-1,2,4-trimethylcyclohexane											E			
1,1-dimethyl-2,4-di(propan-2-yl)cyclohexane														U
1,1-dimethylcyclopropane	Bl													
Cyclic alkenes														
1-(3-methylbutyl)cyclopentene					Sa									
1,3-ditert-butylcyclopenta-1,3-diene					Sa									
1,5,5-trimethyl-3-methylidenecyclohexene					Sa									
1-methyl-3-(2-methylprop-1-enyl)cyclopentane					Sa									
1-methyl-3-(2-methylprop-2-enyl)cyclopentane					Sa									
Linear alkanes														
butane		Br				Sk					E			

(*continued on next page*)

Table 5 (*continued*)

Sources	**Ante-mortem (AM) sources**										**Post-mortem (PM) sources**			
Compounds	Blood	Breath	Finger nails	Hair	Saliva	Skin	Sweat	Urine	Whole body	Not specified	Early	Middle	Late	Not specified
decane	Bl	Br				Sk					E		L	U
docosane					Sa	Sk								
dodecane	Bl	Br		H	Sa	Sk			WB		E			
dotriacontane											E			
Linear alkenes														
but-2-ene						Sk								
dec-1-ene	Bl										E			
dec-3-ene					Sa									
dodec-1-ene	Bl				Sa	Sk					E			
dodec-2-ene					Sa									
Linear alkynes														
prop-1-yne	Bl													
Ketones														
(2-hydroxy-4-methoxyphenyl)-phenylmethanone						Sk								
1-(2-methylcyclopentyl)ethanone					Sa									
1-(3,5,5,6,8,8-hexamethyl-6,7-dihydronaphthalen-2-yl) ethanone						Sk								
1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphthalen-2-yl) ethanone					Sa									
1-(5-methylfuran-2-yl)ethanone					Sa									
Nitrogen-containing														
(2S)-2-(2-aminopropanoylamino)-4-methylpentanoic acid						Sk								
1,2,3,6-tetrahydropyridine						Sk								
1,3,5,7-tetrazatricyclo[3.3.1.13,7]decane											E	M		U
1,3,7-trimethylpurine-2,6-dione					Sa	Sk								
1,3-diethyl-1,3-diphenylurea										U				
Sulfur-containing														
(ethyldisulfanyl)ethane (DIETHYL DISULFIDE)													L	
(methyldisulfanyl)ethane											E		L	
(methyldisulfanyl)methane (DIMETHYL DISULFIDE)					Sa		Sw	U			E	M	L	U
(methylpentasulfanyl)methane (DIMETHYL PENTASULFIDE)														U
(methylsulfanylmethyldisulfanyl) ethane													L	
Terpenes														
Monoterpenes														
(4R)-1-methyl-4-propan-2-ylcyclohexene (MENTHENE)											E			
1,1,2-trimethyl-3,5-bis(prop-1-en-2-yl)cyclohexane					Sa									
1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane (EUCALYPTOL)		Br			Sa	Sk					E			
1,4,4-trimethyltricyclo[6.3.1.02,5] dodec-8-ene					Sa									
1,7,7-trimethyltricyclo[2.2.1.02,6] heptane					Sa									
Sesquiterpenes														
(1R,2S,6S,7S,8S)-1,3-dimethyl-8-propan-2-yltricyclo [4.4.0.02,7]dec-3-ene (COPAENENE)					Sa									
(1R,5S,6R,7S,10R)-10-methyl-4-methylidene-7-propan-2-yltricyclo[4.4.0.01,5]decane (BETA-CUBEBENE)					Sa									
(1S,2R,5S,7R,8R)-2,6,6,8-tetramethyltricyclo[5.3.1.01,5] undecan-8-ol (CEDROL)								U						
(1S,4aR,8aR)-7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene (GAMMA-CADINENE)					Sa									
(1S,4aR,8aS)-4,7-dimethyl-1-propan-2-yl-1,2,4a,5,8,8a-hexahydronaphthalene (BETA-CADINENE)					Sa									
Diterpenes														
3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene						Sk								
Triterpenes														
2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene (SQUALENE)		Br				Sk								
Norterpenes														
2,6,10,14-tetramethylpentadecane (PRISTANE)														
Terpenoids														
Monoterpenoids														

(*continued on next page*)

Table 5 (*continued*)

Sources	Ante-mortem (AM) sources										Post-mortem (PM) sources			
Compounds	Blood	Breath	Finger nails	Hair	Saliva	Skin	Sweat	Urine	Whole body	Not specified	Early	Middle	Late	Not specified
(1R,2S,5R)-5-methyl-2-propan-2-ylcyclohexan-1-ol (MENTHOL)						Sk					E			
(2R,5R)-5-methyl-2-propan-2-ylcyclohexan-1-ol (NEOMENTHOL)											E			
(2S,5R)-5-methyl-2-propan-2-ylcyclohexan-1-one (MENTHONE)											E			
(3R)-3,7-dimethyloct-6-en-1-ol (CITRONELLOL)			F	H										
1,7,7-trimethylbicyclo[2.2.1] heptan-2-ol (BORNEOL)					Sa									
Sesquiterpenoids														
(1S,2R,6R,7R,8S)-1,3-dimethyl-8-propan-2-yltricyclo [4.4.0.02,7]dec-3-ene (YLANGENE)					Sa									
1,1,2b-trimethyl-6-methylidene-2,2a,3,4,5,6a,7,7a-octahydrocyclobuta[a]indene (BETA-CLOVENE)					Sa									
1,6-dimethyl-4-propan-2-yl-1,2,3,4-tetrahydronaphthalene					Sa									
1-methyl-5-methylidene-8-propan-2-yltricyclo[5.3.0.02,6] decane (BETA-BOURBONENE)					Sa									
2,2,7,7-tetramethyltricyclo[6.2.1.01,6]undeca-3,5,9-triene (ISOLONGIFOLENE)					Sa									
Diterpenoids														
(3S)-5-[(1S,4aS,8aS)-5,5,8a-trimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]-3-methylpent-1-en-3-ol (13-EPIMANOOL)						Sk								
7,11,15-trimethyl-3-methylidenehexadec-1-ene (NEOPHYTADIENE)														U
Others														
(2R)-2,5,7,8-tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol (VITAMIN E)						Sk								
(3S,5S,9R,10S,13R,14R,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,5,6,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol (LATHOSTEROL)						Sk								
(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol (CHOLESTEROL)						Sk								
(4-hydroxyphenyl)phosphonic acid												M		
(8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,8,9,11,12,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3-ol						Sk								

after death due to autolysis and putrefaction. Knowledge of the transition from ante- to post-mortem scent is significant in search operations, as each team of detection dogs is trained to locate either living individuals (search-and-rescue dogs) or human remains (human remains detection dogs), but not both. Knowing when to deploy each canine type can be a challenging and costly endeavour when the status of the victim (s) is unknown, and search-and-rescue (SaR) teams will deploy search dogs depending upon the likelihood of locating survivors. Gaining an understanding of the transition from a living to deceased scent is therefore valuable when locating missing persons or mass disaster victims, as it can increase search efficiency and thus the chance of survival.

One avenue to decipher the transition from ante- to post-mortem scent is to directly compare the prevalent VOCs. However, the living and deceased volatilomes are dynamic and complex, with differences existing in the number of compounds, prevalence of individual VOCs, and the ratio of classes present. Table 5 presents an excerpt of the ante-mortem and post-mortem volatiles reported in literature from 2010 onwards, with the full list presented in the supplementary materials in Table S1. For a more accurate comparison of these VOC profiles, only post-mortem studies conducted using human cadavers have been included [111,112,12,13,6,71,98] in Table S1, and for live scent only VOC studies on healthy individuals have been used, as the presence of disease or infection has a definitive effect on an individual's VOC profile [10,46,119,146].

Carbonyl-containing compounds such as carboxylic acids, ketones, aldehydes, and esters are commonly reported within both the ante- and post-mortem volatile profiles [12,30,54,71]. Linear ketones such as acetone, 2-butanone, 2-hexanone, and 2-heptanone are detected in ante-mortem matrices such as breath, skin, and urine [30,48,162], and other ketones such as branched compounds (4-methyl-2-pentanone, 3-methyl-pentanone, 2-methyl-3-pentanone, 6-methylhept-5-en-2-one) cyclic ketones (cyclopentanone), and diketones (2,5-hexandione) have been heavily reported in breath and skin studies [16,23,69]. In post-mortem profiles, ketones such as acetone, 3-propanone, and 2-pentanone [111,163,164,71,98] are produced via the decomposition of carbohydrates and during the aerobic oxidation of unsaturated fatty acids [112]. Acetone is formed during lipolysis and lipid peroxidation, and is derived from pyruvate through the decarboxylation of acetoacetate [112]. The ante-mortem production of acetone has been related to several pathways, including the decarboxylation of Acetyl-CoA, the oxidative

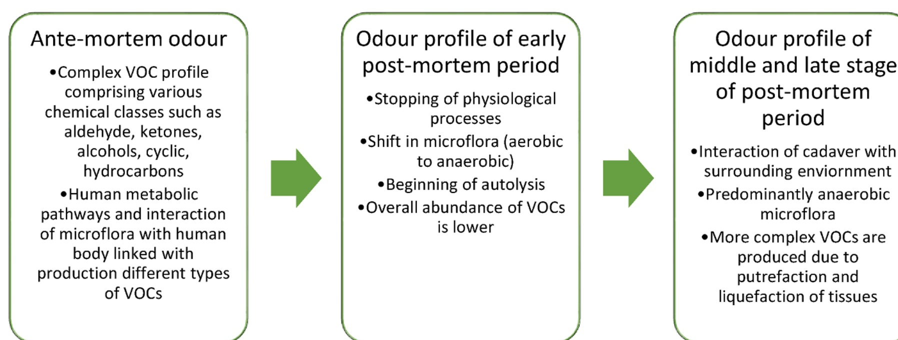

Fig. 2. General trend seen in the transition from ante-mortem, early, middle and late-stage post-mortem odour.

Table 6
Number of compounds per class found in ante-mortem studies, displayed as a colour scale within each sampled matrix. Green represents a lower number of compounds, where red represents a higher number of compounds.

Classes	Blood [31-33]	Breath [10, 16-17, 32-33, 42-45]	Finger nails [41]	Hair [41]	Saliva [18-20, 32-33, 41]	Skin [14, 21-23, 32, 41, 43, 45, 65-70, 122-124, 147-148, 154]	Sweat [39-40]	Urine [4, 32-38]	Whole body [22, 24-28, 43, 45, 99-100, 125]
Alcohols									
Aldehydes									
Aromatics									
Carboxylic acids									
Esters									
Ethers									
Halogenated									
Hydrocarbons									
Ketones									
Nitrogen-containing									
Sulfur-containing									
Terpenes									
Terpenoids									
Others									

degeneration of squalene of human skin, 2-propanol metabolism, and diet [48]. Acetone is heavily reported in both living and deceased human studies (Table S1), although it is notably absent from the early and middle stages of decomposition. This could be due to a lack of research within the area, however it could also be attributed to its degradation pathways. The processes of lipolysis and peroxidation occur in the presence of water and oxygen respectively, which require tissue liquefaction and an aerobic environment. These steps do not occur until

active decay, which can be classed as middle-stage decomposition depending on the deposition environment. Further investigation into the presence of acetone is therefore required, as its presence could be indicative of life during initial rescue efforts.

Additional carbonyl-containing compounds have also been identified in ante-mortem scent, including hexanal, heptanal, octanal, nonanal, and decanal from exhaled breath and skin (Table 3 and Table S1). Benzaldehyde is frequently detected in the headspace of breath, skin, and urine, and it is hypothesized that benzaldehyde is a by-product of toluene breakdown in the body [162]. The studies conducted on post-mortem decomposition odour have also identified these VOCs during the early post-mortem period. The aldehydes hexenal, nonanal and decanal have been detected in post-mortem studies [71,112], and, like ketones, are produced through the aerobic oxidation of unsaturated fatty acids [52,71,95]. During the early post-mortem period, linear aldehydes are more prevalent, and have been found in the headspace of adipose tissue, muscle tissue, and bone [54,111,137]. Oxygenated species within the profiles of living and post-mortem odour share similarities during the early post-mortem period, in terms of the straight-chain compounds detected and identified. The main difference in ante-mortem and post-mortem VOC profiles for the ketones and aldehydes are the number of compounds and the different types of branched and saturated (Table 5 and Table S1). Unsaturated aldehydes such as dec-2-enal, hept-2-enal, non-2-enal, and oct-2-enal are frequently reported in ante-mortem studies of saliva, skin, and whole-body emissions, and branched aldehydes and ketones are also commonly seen in living studies. In comparison, saturated compounds including hexanal, nonanal, and propanal have been found in both ante- and post-mortem studies. Although saturated aldehydes are reported from both living and deceased individuals, it is important to note that the scarcity of VOC research on human remains skews the comparison, and manipulates the data to favour ante-mortem emissions.

Short-chain alcohols such as methanol, ethanol, 1-propanol, 1-butanol, and 2-butanol are reported in live scent [22,24,26], and branched alcohols such as 2-methyl-1-propanol have been detected in faeces, urine, breath, and skin [162]. Unsaturated alcohols including 2-propen-1-ol, 2-buten-1-ol, 1-octen-3-ol and 2-octen-1-ol have also been identified from skin [162], as have phenols and benzyl alcohol [26]. In ante-mortem contexts, acid reduction in the gastrointestinal tract, glycolysis, and the breakdown of pyruvate and citrate leads to the generation of these alcohols [30], and studies have found them in the headspace of faeces, breath, and blood (Table S1). In post-mortem contexts, alcohols are created through the decomposition of many macromolecules and different tissue types. For example, carbohydrates will decompose to form oxygenated compounds such as short-chain alcohols in anaerobic conditions [52,155], glycerol sourced from lipids will produce short-chain alcohols, and the amino acids found in muscle tissue will degrade to alcohols and acids [165]. In the early post-mortem period, studies have not detected branched alcohols or unsaturated alcohols in higher abundance (Table S1), and the most consistently reported alcohols have been short, straight-chain species such as ethanol and 2-propanol [95,112]. Phenol has also been detected in the early, middle, and late post-mortem periods [111,112,161,71,86,89].

Within living matrices sulfides are primarily present in urine and faecal studies [4,62], with only one saliva study reporting their presence [18] (Table S1). The study on urine was performed by Mochalski *et al.* [4], who analysed the volatiles of human urine before and after four days of storage in a room-temperature environment. The aim of this

Table 7
Number of compounds per class found in post-mortem studies, displayed as a colour scale within each sampling period. Green represents a lower number of compounds, where red represents a higher number of compounds.

Classes	Early [71, 86, 95-96, 113, 128, 161]	Middle [71, 86, 95-96, 113, 129]	Late [83, 85-86, 90, 96, 113]	Unspecified [89]
Alcohols				
Aldehydes				
Aromatics				
Carboxylic acids				
Esters				
Ethers				
Halogenated				
Hydrocarbons				
Ketones				
Nitrogen-containing				
Sulfur-containing				
Terpenes				
Terpenoids				
Others				

study was to find biomarkers of entrapped disaster victims, and dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) had the highest increase (five-fold) of any sulfides present in urine. The presence of sulfur species within ante-mortem urine matrices can be the result of several origins, however their endogenous production is ascribed to the metabolism of methionine and cysteine in the transamination pathway [4]. Methanethiol is another sulfide present in urine [4], and is produced through the enzymatic metabolism of methionine. Methionine can be oxidised to form DMDS or undergo methylation to form dimethyl sulfide, can further undergo oxidation to form dimethyl sulfone [4]. Sulfides in post-mortem studies have generally been reported to appear during the bloat stage of decomposition, however several studies have detected them during the fresh stage [111,113,128,129,83,95]. DMDS and DMTS are commonly present in decomposition due to the aerobic and anaerobic microbial decomposition of sulfur-containing methionine and cysteine [120], and are the most heavily reported sulfur-containing compounds within the post-mortem literature (Table S1). Despite the presence of DMDS and DMTS in aged urine, their ubiquitous nature within the post-mortem odour profiles limits their use as ante-mortem biomarkers. This shows that other compound classes should be the focus of such research, with future directions directly comparing ante-mortem and early post-mortem volatiles.

Hydrocarbons are among the most reported compound class in living human scent studies and post-mortem odour, and within this class, linear species, aromatics, and branched hydrocarbons are the more commonly reported compound types. In living scent, the detection of these hydrocarbons is attributed to the breakdown of unsaturated fatty acids via peroxidation, and the detection of aromatic hydrocarbons is associated with smoking in breath samples [162]. Isoprene is a commonly detected compound from breath, skin, urine, and whole-body emissions (Table S1), and with only one study reporting its emission from human remains [94]. The absence of isoprene within other post-mortem profiles could be due to the lack of a synthesis pathway after metabolism has ceased. This highlights its potential as a biomarker for living victims, and further research should be conducted on fresh remains to make this distinction. Hydrocarbons are also one of the most persistent classes of VOCs detected in living and deceased odour profiles [21,112], with linear alkanes and branched alkanes being found across ante-mortem matrices and various post-mortem stages (Table S1). Hydrocarbons are present in breath samples predominantly due to their active or passive ingestion from the atmosphere [22,30], and in a post-mortem setting they are produced through the decomposition of lipids [112]. Researchers have also documented saturated and unsaturated hydrocarbons due to the cutaneous oxidation of sebum components (*e.g.* squalene and fatty acids), which are likely to be present during the early post-mortem period.

The transitional odour profile from ante-mortem to post-mortem scent is a challenging aspect, and there has been extensive research and metabolic pathway data published for the volatiles that could contribute to human scent. This cannot be said about the decomposition odour, as the complex processes leading to post-mortem VOCs production are not entirely understood. Due to ethical restrictions profiling human cadavers is difficult, and analysing human remains in an outdoor environment to simulate disasters or clandestine body disposals is limited to taphonomic research facilities. Researchers have extensively studied and identified volatiles released from human analogues (Table 2), however recent studies have demonstrated that although the VOCs classes are similar, the abundance and ratio of these classes are not comparable to that of human cadavers [6,12]. This new understanding limits the usefulness of studies using animal remains, and highlights the need for further odour research on human decedents.

When investigating the early post-mortem period, the notable trend is that very few compounds from human scent are present (Fig. 2). The second observation is that the profile is comprised of straight-chain alcohols, ketones, aldehydes, and hydrocarbons (*e.g.* 1-propanol, ethanol, hexenal, hexane, and acetone). Other compound types detected are branched VOCs (*e.g.* 2-methyl butanal), followed by unsaturated and aromatic volatiles. After death, most of the metabolic pathways governed by the body stop, and as the cells start to degrade there is a continuous shift in microbial flora. This initial transition in the microbial environment and a depletion of oxygen could lead to fewer VOCs being produced during the early post-mortem period (Fig. 2). As the body's decomposition progresses, the volatile profile becomes more complex, and more compounds and compound classes are observed.

The number of compounds per class found in ante-mortem and post-mortem studies have been presented as a colour scale for ease of interpretation (Table 6 and Table 7). Table 6 indicates that the ante-mortem profile is largely dominated by hydrocarbons, with skin also producing a high level of esters, urine producing ketones, and sweat producing ketones and nitrogen-containing compounds. Hydrocarbons are also produced heavily during decomposition (Table 7), with middle decomposition producing aldehydes and late decomposition producing nitrogen and sulfur-containing compounds. When comparing profiles, it is important to note that studies typically only report the names of detected volatiles, and do not include the abundance of volatiles within the profile or any additional quantification. This further complicates the comparison between ante- and post-mortem profiles, as the intensity of volatiles is a vital aspect of the volatilome, and a high number of compounds in one compound class does not fully reflect the reality of the volatilome.

Further research is therefore required into the volatile profile transition from ante-mortem to peri- and post-mortem states, including reporting the intensity of compounds and establishing methods of quantification. Investigating the concentrations of isoprene and acetone during life and comparing these compounds to early decomposition could aid in locating the living, as the concentration of both compounds seems to drop dramatically once death has occurred. The standardisation of analytical methods between ante-mortem and post-mortem studies would benefit this field, as ante-mortem studies collect passive headspace samples whereas much of the decomposition literature uses sorbent tubes and active collection (Table 1 and Table 2).

In addition to this, the use of GC×GC-TOFMS over GC–MS would greatly benefit ante-mortem volatile profiling, as it would eliminate potential coelution and enable a more accurate assessment of VOCs to be performed. Moving towards using GC×GC-TOFMS for ante-mortem research would also standardise methods between ante- and post-mortem volatile analysis, which would allow for a better comparison of the volatile profiles and allow for the establishment of a comprehensive human odour profile.

6. Conclusion

VOC profiles are complex, dynamic mixtures that are constantly changing, which makes the task of determining suitable biomarkers for living victims incredibly challenging. The transition between the odour profiles of living and dead is difficult to ascertain, as ante-mortem samples have largely been analysed using GC–MS, whereas post-mortem research has shifted to GC×GC-TOFMS. More extensive studies will enable a database of VOCs detected from whole cadavers to be developed, and make future comparisons between live-scent and post-mortem odour feasible. Ideally, both ante- and post-mortem studies need to be conducted in the same environment, to determine the release and behaviour of VOCs with consistent environmental influences. An emphasis on collecting large numbers of samples during the initial post-mortem period is also recommended, in order to accurately establish the odour transition from living to deceased. Further research into this area is therefore required to standardise sampling methods, instrumentation, and experimental sites to accurately determine which volatiles are produced from victims during disaster scenarios.

CRediT authorship contribution statement

Bridget Thurn: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Alisha Deo:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Darshil Patel:** Writing – review & editing, Investigation, Formal analysis. **Emily Sunnucks:** Writing – review & editing, Investigation, Formal analysis. **Shari Forbes:** Writing – review & editing, Supervision. **Barbara H. Stuart:** Writing – review & editing, Supervision. **Steven Su:** Writing – review & editing, Supervision. **Maiken Ueland:** Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. MU is supported by the Australian Research Council DECRA (DE210100494).

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

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